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Morphological and cytochemical studies on the development of the
cestode *Diorchis ransomi* Schultz 1940

Morfologiczne i cytochemiczne badania nad rozwojem tasiemca
Diorchis ransomi Schultz 1940

The present paper deals with the successive developmental stages of *Diorchis ransomi* Schultz 1940 by using the morphological and cytochemical techniques.

Polish parasitology has its traditions in cytological studies on the embryogeny and larval development in the works of Janicki (1906, 1907, 1909, 1921), Wiśniewski (1928, 1930), and Michajłow (1932, 1933). However, the histochemical methods, which are now being introduced ever more widely in developmental studies, were not applied in those works.

These methods, thanks to staining by certain chemical compounds, are of great help in identifying exactly the different cells observed in the development of tapeworms. Another advantage of the histochemical methods is that they greatly facilitate comparison of the results obtained by various authors from different parasitological material.

The techniques used in the present work were those that have been employed many times in this kind of investigation and which have given relatively good results, i.e. methods revealing nucleic acids and glycogen. As there was very little data available, some modifications of the techniques had to be made on some occasions in order to obtain the proper variation for the material examined.

It should be pointed out that *Diorchis ransomi* is not very favourable material for development research. The small dimensions of both the adult tapeworm and the larvae give rise to many technical difficulties in preparing the slides. Besides, the very small dimensions of the cells observed make it very difficult to interpret the material examined. Still, this species has been selected as the continuation of the authoress' previous studies (Rybicka 1957a, b).

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Materials and methods

Diorchis ransomi belongs to the family *Hymenolepididae*; a detailed description of the adult tapeworm was given in a previous paper (Rybicka 1957a). In Poland, the main host of *D. ransomi* is *Fulica atra* L.; it was found relatively often in *Gallinula chloropus* (L.) and, sporadically, in some species of *Anseriformes* (Rybicka 1957a, 1958, Czapliński 1956). The intermediate hosts are in Poland *Ostracoda*: *Cypridopsis vidua* Müll. and *Cyclocypris laevis* Müll. (Rybicka 1957a, 1957b, Jarecka 1958, 1960).

Tapeworms were obtained from *Fulica atra*. The birds were opened not later than 5 hours after death. Entire living worms with gravid proglottides were cut into small strips and fixed in Carnoy's. They were dehydrated in alcohol, cleared in benzene and embedded in paraffin. Serial sections — transverse of some specimens and longitudinal (frontal) of the others — were cut five microns thick.

The eggs were removed from the gravid terminal proglottides in order to prepare total preparations and to study the larval development.

The in toto slides were prepared as follows: the eggs in drops of water were placed on slides coated with an albumin-glycerine mixture. As the water dried, the slide was immersed in 75% alcohol. The eggs, fixed in this way, remained stuck to the slide.

The remaining eggs were fed with *Cypridopsis vidua* Müll. Then on successive days the developed larvae were pressed out of the hosts and placed in drops of water on slides coated with an albumin-glycerine mixture. The larvae were fixed by using the same technique as in the egg fixation. Some specimens were stained directly after fixing, the other ones were kept dry on the slides to be stained later. However, this method of keeping the slides is not very lasting. They kept well for two months but after more than two months the larvae on the slides began to decompose.

Staining techniques

A. Cut-staining technique.

Cytological methods: The following cytological methods were applied: Mayer's hemalaun-eosine staining, Heidenhein's hematoxylin (according to Romeis 1948) and Prenant's trichrome. In addition, methyl green-pyronin (according to Unna) was used for observation.

Cytochemical methods: Several nucleic acid reactions were applied: (1) for DNA determination the Feulgen technique was used, both without additional staining and with light green staining; (2) for RNA determination methyl green-pyronin staining according to Brachet's method was applied. Several modifications of this method were used: according to the methods of Pearse 1953, Godlewski et Vorbrodt 1954, and Jordan et Baker 1955. For control purposes the preparations were incubated in ribonuclease; (3) for DNA and RNA determination toluidine blue staining was applied. With this method, HCl hydrolysis of the preparations (according to Vendrely 1950) was used in addition to check the presence of RNA.

Besides, the periodic acid-Schiff reaction (PAS) was also used to determine the polysaccharides. With the PAS reaction the saliva control was used to determine glycogen.

Not all of the methods applied gave satisfactory results, but it must be emphasized that comparison of the preparations stained by several different methods made it much easier to analyse the embryonic development.

Special mention is due to Prenant's trichrome method as it made it possible to distinguish the various organs of the tapeworm. This proved particularly valuable

in examining the Mehlis' gland which could not be distinguished in the carmine stained in toto. The cytoplasm of the glandular cells was stained with light green while the cytoplasm of other cells was stained with eosine.

As regards the cytochemical methods, the Feulgen technique proved to be very useful especially in examining embryonic development. The very small dimensions of the cells and their crowding in some development stages made it impossible to establish their exact number by other methods. With the Feulgen technique the only compound stained was the DNA present in the nucleic chromatine, making it possible to establish the number of nuclei and consequently the number of cells at different stages of embryonic development. Moreover, this technique, which shows the differences in structure of the various nuclei, makes it possible to define the various types of cells and the condition of the cells during development.

On the other hand, it should be emphasized that great difficulties were experienced in applying the Brachet technique for detecting RNA in the material examined. Although many attempts were made to apply different modifications of this method it was only in a few cases that a clear picture was obtained (mainly using Pearse's 1953 modification). But in spite of these difficulties, comparison of these preparations with preparations stained with toluidine blue gave some valuable material for detecting changes in the RNA distribution.

The PAS reaction made possible accurate observation of the changes in glycogen distribution during embryonic development. A detailed study of this problem has been published separately (Rybicka 1960).

B. Procedure for eggs and larvae.

The Vogel 1929 methyl green-pyronin method was mainly used for staining in toto eggs and larvae, as well as a modification of Brachet's method according to Jordan et Baker 1955. The Feulgen technique was also applied.

Staining with methyl green-pyronin method did not give very good results as with the cuts. It should be mentioned that the author used the Vogel's method successfully in other studies to stain coracidia of *Diphyllbothrium latum* and *Triaenophorus lucii*. These experiments have shown that this method is not so useful for staining *Diorchis ransomi* (and maybe for cyclophyllidean cestodes in general) as it is for staining pseudophyllidean coracidia.

Technique of embryogenetic studies

Embryogenetic studies were made after the reconstruction of the embryos from the serial cuts of the gravid proglottides. The illustrations to this paper show all the serial cuts of each embryo, so every stage is represented by several drawings. This technique was not used in any of the papers on tapeworm embryogeny known to the author. The reconstruction of the embryo made it possible to observe the changes in the number of cells during development and was particularly useful in studying the final condition when the cells were densely crowded.

GAMETOGENESIS

Morphogenesis of the reproductive system

Spherical or oval testes about 60 μ in diameter appeared in the youngest proglottides of *D. ransomi* when the strobila was about 400 μ wide; cirrus sac was also visible. In older proglottides, when the strobila was about 650—900 μ wide, the testes reached full maturity and produced sperm. Two short ducts joined and formed a common duct leading to the vesicula seminalis externa. The cirrus sac was then fully formed with the vesicula seminalis interna. At this stage both vesiculae seminales contained sperm. The vagina and a large receptaculum seminis filled with sperm were observed at this time.

The female reproductive glands (ovary and vitellaria) were in the initial phase of development at this stage. The diameter of the various lobes of the ovary was 50—65 μ . Then the Mehlis' gland began to appear between the ovary and the vitellaria. In the further proglottis, when it was 1000—1020 μ wide, the testes began to disappear. The structure of the spermoducts did not undergo any change, but the female glands reached full maturity. The width of the ovary reached 350 μ and that of the vitellaria — 100 μ . The width of the Mehlis' gland was 85—95 μ . When the female glands reached maturity, the uterus appeared in the proglottis. In the gravid proglottides, the uterus containing embryos filled the entire proglottis. The ovary and vitellaria gradually disappeared.

Cytogenesis of the reproductive elements

Spermatogenesis. In immature testes of *D. ransomi* two types of cells could be distinguished: (1) small cells (2.5 μ in diameter) with small, spherical, strongly basophile nuclei, and (2) large cells (diameter 5 μ) with slightly basophile nuclei with chromatin network. The Brachet reaction showed the presence of RNA in the cytoplasm. At this phase the tubules were not yet clearly visible.

In the older proglottides, apart from the above-mentioned cells, new agglomerations of cells resembling them in structure but smaller could be clearly seen. At this phase bunches of spermatozoa were also visible in the testes. Besides, there were also agglomerations of colourless spherical shapes resembling cells but without nuclei. They did not contain any Feulgen-positive elements. The lumen of the tubules could be seen between the described cells.

In the spermatozoon it was possible to distinguish the head which was a little thicker (diameter about 0.5 μ) and the thin tail which may be as long as about 8 μ . All the spermatozoa were basophile. Both the head and the tail were Feulgen-positive and stained green after Brachet reaction. This reaction showed the presence of DNA in the entire spermatozoon and not only in its head. It is interesting to note that toluidine blue did not give any reaction.

PAS positive materials were not found in the testes or spermatozoa.

Oogenesis. The young ovary of *D. ransomi* was filled with spherical cells with large basophile nuclei. As the ovary developed these cells grew, their diameter reaching about 20 μ ; the diameter of their nuclei was about 10 μ . During the development of the oocytes their nuclei became much less basophile. They did not take the stain very well showing the presence of a small number of strongly basophile chromatin granules. There was always a large (about 1.7 μ), strongly acidophile nucleolus in the nuclei of the oocytes. Among the chromatin granules observed in the nucleus it was always possible to distinguish one definitely larger concentration of „nucleolus associated chromatin” after the application of the Feulgen technique or toluidine blue. The cytoplasm of the oocyte contained a large concentration of acidophile granular substance. The Brachet technique and staining with toluidine blue showed that the cytoplasm of the oocyte was rich in RNA. The PAS reaction showed the presence of polysaccharides in the cytoplasm of the oocyte. This method gave a diffuse reaction and morphological granules could not be distinguished.

During the development of the oocytes in the ovary meiosis was not observed.

Observation of fertilization was rather difficult. Although a large number of sections were examined and the mature segments of the cestode reconstructed, I did not see any oocytes on their way from the ovary to the uterus*. Meiosis in the ootype after it had entered the uterus was not observed either.

In some cases the presence of two large pronuclei with definitely acidophile nucleoli was observed in early embryos, which confirms the observations of other authors.

Vitellaria. The vitellaria of *D. ransomi* were filled with homogeneous spherical cells, their diameter being about 4 μ . The nuclei of these cells were strongly basophile. Using toluidine blue stain, I obtained a definite metachromatic reaction in some cases and in others green staining of the nuclei of the vitellary cells. The Brachet reaction showed the cytoplasm of these cells to be rich in RNA.

Mehlis' gland. The Mehlis' gland was composed of large cells as long as 20 μ . The spherical weakly stained nuclei were 3.5 to 4 μ in diameter. The structure of the gland could be seen particularly well after Prenant's trichrome staining. The cytoplasm of the cells took the light green stain very well, while other cells took the eosine stain. With the application of Brachet's method, the cytoplasm of the gland cells remained almost colourless, which showed that they were poor in RNA. The nuclei of these cells took an intensive green colour.

The Mehlis' gland gave an intensive PAS-positive reaction. The stain did not become less intense after saliva control and this pointed to a big concentration of non-glycogenous polysaccharide.

Discussion

In the studies on the gametogenesis of *D. ransomi*, the question of spermatogenesis should be dealt with more extensively. As previously mentioned, this problem is very little known. Govaert 1960 has published a paper on his cytological and cytochemical studies of gametogenesis in *Fasciola hepatica*. Although these studies concern a trematode, my observations of *D. ransomi* have so much in common with those of Govaert that a comparison can be made with the results he obtained and his interpretation of them. It should be emphasized that it is much more difficult to make such an analysis with the cestode species studied than with the *Fasciola hepatica*, owing to the much smaller dimensions of the cells and entire organs. This

* I would like to refer to the papers of Weizman (1939, 1953 a, 1953 b), who studied the development of the female reproductive system in two species of *Taenia*. He came to the conclusion that the reproductive glands of *Cyclophyllidea* were rudimentary organs and could not produce eggs capable of development; the embryo was formed in the uterus from its epithelium. This theory was put forward on the basis of the fact that he never found eggs in the oviduct, ootype or uterine duct. There is no doubt that Weizman's theory is incorrect. It is not probable that spermatogenesis and oogenesis could take place in such a typical way in rudimentary organs. Comparison of the oocytes from the ovary and uterus revealed that they were undoubtedly the same cells.

explains why I was not able to make such a detailed observation of spermatogenesis as was possible with the *F. hepatica*.

In the young testes of the *F. hepatica*, Govaert distinguished small cell nuclei forming the wall of the tubules as well as larger cell nuclei, which he described as spermatogonia. As the testes develop, the spermatogonia form rosette-like agglomerations in which spermatogenesis takes place synchronically. The successive meiosis create spermatocytes of the I and II order, which in turn develop into a cigar-shaped spermatid with spherical nuclei and a thick cytoplasm. In this cytoplasm two layers can be differentiated, the outer one being eozynophile, rich in RNA, and the inner one, which remains colourless when eosine and the Unna-Brachet method is used. It has no RNA, but has protein rich in histone. As the nuclei develop, the spermatids become long and tail-like, pierce through the layer of cytoplasm and emerge. In this way spermatozoa are formed. The spermatozoon head has a small cap formed from the inner layer of colourless cytoplasm. The rest of the cytoplasmic spermatids without nuclei remain in agglomerations and then decompose.

Comparison with the Govaert's observations led to the conclusion that the small nuclei observed by me in the first stage of the testes development correspond to the cells described by Govaert as forming the walls of the tubules, while the large cells observed by me at this stage correspond to the spermatogonia he described. In further development stages I have observed agglomerations of large cells, which were probably spermatocytes and spermatids in various stages of development, forming the characteristic rosette-like agglomeration in the *F. hepatica*. Govaert's studies also give an explanation of the origin of the colourless cell agglomerations which are undoubtedly the remains of the cytoplasmic spermatids after the spermatozoa have left them. In most cases a bunch of spermatozoa was visible near them. The DNA content observed in the entire spermatozoa confirmed Govaert's observations and pointed to the fact that in *D. ransomi*, as in *F. hepatica*, the entire spermatozoon was of nucleic origin. I am not able to determine whether there was a small cytoplasmic cap on the head of spermatozoon because the technique applied did not show (as in Govaert's data) the colourless protein compounds found there.

A point worth mentioning is that the spermatozoa remained colourless after toluidine blue, while at the same time they definitely contained DNA, as was shown by the Feulgen and Brachet techniques. This problem calls for additional cytochemical studies.

Comparison of my observations on spermatogenesis with Govaert's data leads to the assumption that this process takes place in the same way in cestodes as in trematodes. This question can only be settled after studying a larger number of species.

Among other observations concerning the cytochemical structure of the reproductive glands of *D. ransomi*, stress should be laid on the presence of non-glycogenous polysaccharide in the Mehlis' gland. This observation confirms the results of the new histochemical studies of this gland made so far. A strong PAS-positive reaction has been observed in several other species of cestodes (Smyth 1956, Johri 1957, Hedrick et Daugherty 1957) and in trematodes (Johri et Smyth 1955 — quoted from Smyth et

Clegg 1959; Rao 1959). The attempts made so far to determine the chemical composition of this substance (Smyth 1956, Johri 1957) using the method described by Pearse 1953 for vertebrates, gave negative results, showing that it was not any of the four groups of PAS-positive polysaccharides discovered so far.

EMBRYONIC DEVELOPMENT

One of the characteristic features of the oncosphere is the presence of two types of cells which differ in size and structure. They were first described by Vogel 1929 in the coracidium of *Diphyllbothrium latum*; the small cells were called „somatic cells”, while the large ones „plastin cells”. Wiśniewski 1930 also found the embryo of *Archigetes* to consist of two types of cells calling the small ones „somatic” and the large ones — „germinative” cells.

In papers published so far on the embryonic development of cestodes, ones attention is drawn by the relative lack of information on cytological changes taking place in the final stages of this development before the formation of the oncosphere. The early stages of development have been studied very thoroughly by many authors. In most cases accurate observation was made of the development of the various cells up to the 18—20 blastomere stage, after which a further cell multiplication made observation very difficult. Most authors have therefore described the differentiation of cells without counting them and then go on to describe in detail the morphology of the oncosphere.

In this paper special attention is given to the changes taking place in the final stage of embryonic development, about which the least is known.

Early embryo

The first oocytes appear in the uterus in the initial stage of its development. The number of embryos gradually increases, which could be seen during observations of over a dozen successive gravid proglottides. During the formation of the uterus and the appearance of the first embryos there, an irregular granular substance could be seen that disappeared later as the embryos developed.

The oocytes entering the uterus were a little different from those observed in the ovary. In their cytoplasm the acidophile granules previously observed disappeared. A large, spherical, strongly basophile nucleolus could be seen in the nucleus. After Feulgen technique, the nucleus of the oocyte showed very little DNA, concentrated in the form of a few chromatin granules, as well as one larger concentration of „nucleolus associated chromatin”.

A single vitelline cell entered the uterus together with the oocyte and visible changes also took place in the cell at this stage. The basophilia of the nucleus was much weaker than that previously observed in the vitellary. The nucleus had a definitely chromatin structure. The PAS reaction and the saliva test showed a large concentration of large glycogen granules in the vitelline cell. This concentration was considerably larger than that previously observed in the vitellary. This glycogen was the only PAS-positive material in the embryo and uterus of this cestode.

The material studied did not show the presence of spermatozoa in the egg cell.

After penetration into the uterus, the oocyte together with the vitelline cell was surrounded by a delicate, almost invisible membrane irregular in shape. It was difficult to define exactly its origin. The changes observed in the structure of the oocyte and vitelline cell after their penetration into the uterus showed that this membrane could be the product of both these cells. The disappearance of the granular material in the uterus observed at this stage would point to the fact that it could take part in the formation of the membrane.

Cleavage in *D. ransomi* is total and unequal. The first cell divisions did not take place simultaneously in all embryos because those that penetrated to the uterus earlier started cleavage at once while the newly fertilized eggs were still entering the uterus. All the observed cell divisions took place by mitosis. The small dimensions of the nuclei and chromosomes made it impossible to watch all the mitotic figures. Figures corresponding to metaphase and anaphase were observed. The number of chromosomes could not be exactly determined on the very thin cuts ($5\ \mu$). It can be assumed that $1n = 8$, which would agree with Spätlich's data (1925) on *D. inflata* (according to Jones 1945, in *Diorchis reynoldsi* Jones and *D. ralli* Jones $2n = 10$).

The first division of the fertilized egg gave two large blastomeres (macromeres) and two small (micromeres). The nuclei of the macromeres were not strongly basophile and contained a few chromatin granules.

At the time when the number of embryo cells reached 18—25* (Fig. 1) three macromeres could be distinguished in it with weak basophile nuclei containing small chromatin granules and a large „nucleolus associated chromatin”. The diameter of the macromere nucleus was $4.5\ \mu$ — $5.5\ \mu$. There

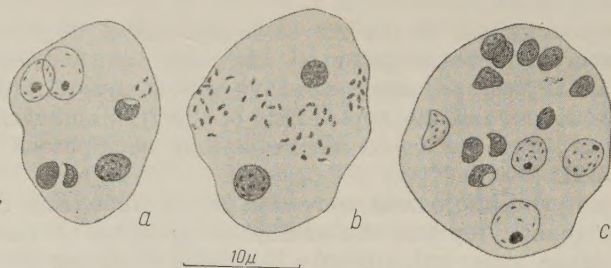


Fig. 1. Early embryo of 28 cells. a, b, c — successive cross sections (Feulgen technique)

were also five to seven mesomeres with weak basophile nuclei and numerous intensively stained chromocenters. The diameter of their nuclei was $2.5\ \mu$ — $3\ \mu$. The third group of cells comprised ten to fifteen micromeres with small ($1.7\ \mu$ — $2\ \mu$), strongly basophile nuclei, in which a polar concentration of chromatin could frequently be observed. This phenomenon can be explained

* I do not give the constant number of cells because this is a phase of continuous cell divisions and consequently continuous changes in their number.

by the great speed of the successive cell divisions as a result of which the chromatin is not evenly distributed over the whole nucleus in the telophase. The cell boundaries were not visible. The Brachet technique pointed to the presence of RNA in the syncytial cytoplasm of the embryo.

At this stage, the embryo was spherical or oval. Three macromeres on the surface separated from the inner cell mass together with a thick layer of granular cytoplasm. The syncytial cytoplasmic layer of the macromeres surrounded the inner cell mass forming the outer membrane of the embryo. The shape of the membrane was very irregular at first, and was thicker where the nuclei were situated.

After the outer membrane formation a great change in glycogen distribution was observed (Rybicka 1960). Previously, the glycogen entering the embryo together with the vitelline cell formed an unchanging single granular agglomeration, but following outer membrane formation all the glycogen passed from the embryo to this membrane, where it appeared in diffuse state. The width of the embryo at this stage was about $20\ \mu$ and the diameter of its membrane about $30\ \mu$.

The embryonic cells enclosed by the outer membrane continued to divide intensively, their number finally reaching 65—70. This was the maximum number of cells observed during the development of *D. ransomi* embryo. At this stage (Fig. 2) the great majority were cells described above as microme-

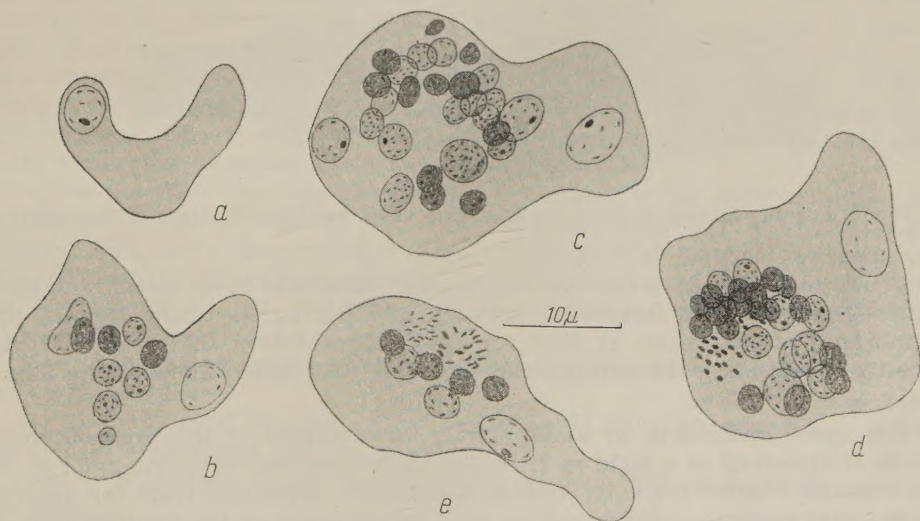


Fig. 2. Early embryo of 70 cells. a, b, c, d, e — successive cross sections (Feulgen technique)

res. An accurate determination of their number as well as the number of the mesomeres was difficult at this stage owing to their dense crowding in the embryo. The previously syncytial cytoplasm of the macromeres concentrated around the nuclei showing the presence of RNA after the Brachet technique.

Following the formation of the outer membrane, great morphological and cytological changes began in the embryo. This development phase was called by me the preoncosphere. It should be pointed out that owing to its intense developmental changes the preoncosphere phase cannot be treated as a definite morphological form, contrary to the oncosphere, which has a constant morphology upon entering the intermediate host.

Preoncosphere

Morphological changes. The first characteristic feature of the preoncosphere of *D. ransomi* was a change in shape. The previously spherical or oval embryo took an elongated spindle-like form (Fig. 3). At the same time its outer membrane also got much longer forming the long filaments characteristic of this species. The length of the embryo at this stage was about $20\ \mu$ and

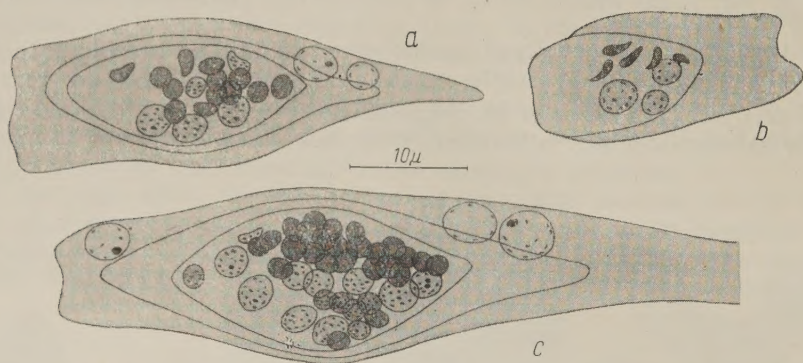


Fig. 3. Preoncosphere. a, b, c — successive cross sections (Feulgen technique)

its width about $13\text{--}15\ \mu$. As previously mentioned, the outer membrane contained glycogen. It is interesting to note that during the elongation of the membrane, the glycogen at first remained concentrated in its inner layer directly surrounding the embryo and was only distributed all over the membrane later.

The inner membrane, or embryophore, was formed at the preoncosphere phase. It appeared as a rigid spindle-like membrane enclosing the embryo. In the material studied the embryophore was clearly separated from the embryo at its polar ends. Cellular structure was not observed in the embryophore of *D. ransomi*.

Cytological and cytochemical processes. One of the most characteristic features of the preoncosphere was a reduction in the number of cells of the embryo. In the previous phase, the number of cells was $65\text{--}70$ in various embryos. The number remained the same in the first period of elongation of the embryo. In its further development the number of cells decreased considerably, finally reaching some 26 in the oncosphere.

The majority of preoncosphere cells were micromeres with strongly basophile nuclei. Their syncytial cytoplasm was granular, the granules containing RNA. Two groups of micromere nuclei were recognized after the Feulgen technique. There was a relatively small number of nuclei with a distinctly chromatin network. The other group comprised very numerous homogeneous nuclei. This homogeneous structure pointed to degeneration processes taking place in these cells (Swanson 1957). At the same time, irregular fragments of decomposed nuclei were observed after Feulgen technique (Fig. 3c).

Simultaneously, changes took place in the syncytial cytoplasm of the micromeres. The basophilia of the cytoplasm decreased and the Brachet reaction revealed the complete disappearance of RNA.

The degeneration of the small nuclei explained the previously observed reduction in the total number of embryonic cells.

The remaining small nuclei with a definite chromatin network were situated in the hooks region of the oncosphere in the form of a syncytial group with cytoplasm completely devoid of RNA.

The other group of preoncosphere cells were those derived from mesomeres. They had larger nuclei which showed numerous chromocenters and a „nucleolus associated chromatin” after Feulgen technique. After toluidine blue, these nuclei were stained a dark blue in the young preoncosphere. The clearly outlined cytoplasmic layer showed very small RNA content after toluidine blue and the Brachet reaction. During the development of the preoncosphere certain changes took place in these cells. Their nuclei became less basophile (after toluidine blue they changed colour from blue to green). The cytoplasmic layer thickened and its basophilia increased considerably. The toluidine blue and the Brachet reaction revealed a large increase of RNA content in the cytoplasm. This cytological structure showed that these were the so-called „plastin cells” of Vogel 1929. The mitotic figures in these cells were sometimes observed at the preoncosphere phase.

After the PAS reaction, red staining appeared in the embryo during the elongation of the preoncosphere, which did not disappear after the saliva control. This was a new non-glycogenous PAS-positive material not observed in earlier stages of development, either in the embryo or in the uterus. After its appearance in the preoncosphere this material remained in the fully formed oncosphere (Rybicka 1960).

The following processes were distinguished as characteristic of the *D. ransomi* preoncosphere:

- (1) morphological changes in the embryo and its outer membrane, giving the typical oncosphere and egg form;
- (2) the formation of the embryophore between the outer membrane and the embryo;
- (3) the degeneration of a part of the micromeres leading to a reduction in the total number of embryonic cells;
- (4) the transformation of the mesomeres into characteristic plastin cells;
- (5) changes in RNA distribution in the embryonic cells (disappearance of RNA in the cytoplasm of the micromeres and its agglomeration in the plastin cells);
- (6) the appearance of non-glycogenous PAS-positive material in the embryo.

Oncosphere

The spindle-like oncosphere of *D. ransomi* (Fig. 4 and 5) was surrounded with a thick rigid embryophore and an elongated outer membrane. The length of the oncosphere was 30–35 μ , its width being 10–12 μ . The length of the embryophore was 65–70 μ , and its width about 15 μ . The outer membrane formed very long filaments which were sometimes as long as 800 μ .

Three large, slightly basophile macromere nuclei were visible in the outer membrane. Diffuse PAS-positive material was present there. Beneath the outer membrane there was a rigid embryophore which did not have a cellular structure. With the staining methods used, the embryophore did not take any

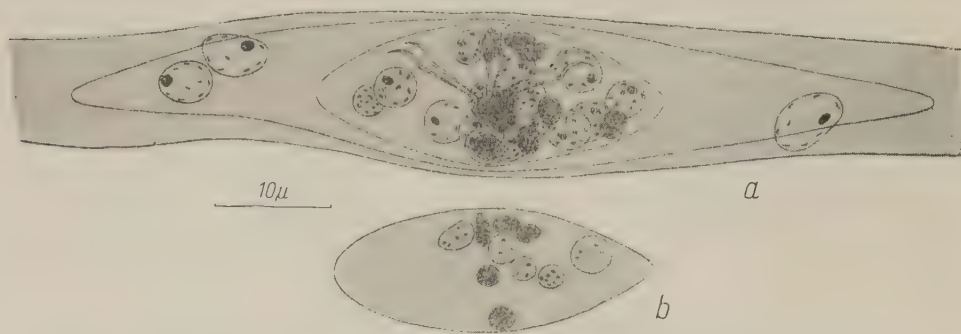


Fig. 4. Oncosphere. a, b — successive cross sections (Feulgen technique)

colour. Under the embryophore, the delicate oncosphere membrane could be seen closely adhering to the embryo. Three pairs of embryonal hooks, about 9 μ long, could be seen. Non-glycogenous polysaccharide was present in the oncosphere.

The oncosphere was composed of about 26 cells. In toto egg preparations showed that their number varied in the various specimens between 23–28. In most of the specimens there were 26 cells. It was very difficult to determine exactly the number of cells in the embryo owing to their very small dimensions and their crowding. The two groups of cells (small cells and plastin cells) were recognized (Fig. 5).



Fig. 5. Oncosphere (Brachet technique)

The number of plastin cells was 12–16. They filled up almost the entire oncosphere except the hooks region. They were relatively large, the diameter of their nuclei being 3–4 μ . The nuclei, which were not strongly basophile,

contained a small quantity of DNA concentrated in a few chromocenters and a „nucleolus associated chromatin” (Fig. 4). The compact, strongly basophilic cytoplasm formed a clearly distinguishable layer around the nucleus. The cytoplasm was rich in RNA (Fig. 5).

Small, syncytial cells, deriving from the micromeres, were confined to the region of the hooks. One little cell on each polar end of the embryo was also observed. The number of small cells in the oncosphere was 9 — 13. Their small (2 μ), spherical or slightly oval nuclei were strongly basophilic and had distinct chromatin network. The nuclei were DNA-rich (Fig. 4). The syncytial cytoplasm of the small cells remained colourless in all staining methods used. The cytochemical reactions showed a complete absence of RNA in this cytoplasm (Fig. 5).

Discussion

The final phase of the embryonic development of *Diorchis ransomi* was called the preoncosphere. This phase corresponded to the „young oncosphere” described by O g r e n 1956 a in *Mesocostoides corti* H o e p p l i. It seems to be right to keep the name oncosphere for the final fixed form of the embryo which does not change until it enters the intermediate host, and to distinguish the earlier phase of development with considerable cytological and cytochemical changes, although its morphology is similar to that of the oncosphere.

One of the main phenomena observed in the preoncosphere phase was the degeneration of the micromeres, causing a reduction in the total number of cells. Among the papers known to the author, the only observation of a reduction in the number of cells during the embryonic development of cestodes was found in the work of J o y e u x et B a e r 1945, devoted to studies of *Catenotaenia pusilla* (G o e z e). The authors compared the species examined with other previous observations and wrote (p. 25): „Notons cependant une particularité qui n’a jamais été signalée chez les Cestodes, ailleurs a notre connaissance. Au stade b de la figure 12* le futur embryon est composé d’une masse cellulaire dont les nombreux noyaux ne possèdent pas tous la même taille ni la même structure. A côté de trois paires de gros noyaux on observe de nombreux noyaux plus petits et se colorant très fortement. Ceux-ci disparaissent dans la suite du développement sans qu’il soit possible de leur attribuer une fonction particulière. La régularité de la taille et de la forme de ces noyaux semble exclure la possibilité de les confondre avec des restes de deutoplasme. Comme d’autre part leur présence est décelée par la réaction de Schiff, leur nature nucléaire ne saurait être mise en doute. Nous espérons éclaircir ce problème par de nouvelles recherches.”

Simultaneously with the degeneration of the preoncosphere micromeres of *D. ransomi*, changes also took place in the structure of its large cells, which changed from syncytial into typical plastin cells. O g r e n 1956 described the changes in the final phase of embryonal development of *Mesocostoides corti* H o e p p l i. In the „young oncosphere” of this species he recognized two groups of cells. Small cells (mesenchyme cell) confined largely to the

* Multi-cell embryo surrounded by a thick cytoplasmic membrane containing three macromere nuclei and the embryophore (K.R. — my explanation).

region of the hooks with granular syncytial cytoplasm were identifiable as the „somatic cells” of Wiśniewski 1930, while the large cells with prominently stained cytoplasmic granules were identified as „germinative cells”. The cytoplasm of both types of cells were stained with pyronin. In further stages of development according to O g r e n ’ s (1956 a) observations, the pyronin reaction nearly disappeared in the cytoplasm of the small cells and the number of their nuclei decreased. In O g r e n ’ s opinion, older oncospheres contained a new type of cells with compact cytoplasm manifesting an intense pyronin reaction, which were identified as V o g e l ’ s (1929) plastin cells. Their number increased and in the oldest oncosphere they were the predominant cells. O g r e n 1956a gives the following interpretation of the phenomena he observed (p. 419): „An exact source of the plastin cells was difficult to discover. It is logical to expect they were produced by mitosis of similar cells multiplying forward into the hook-region. However, mitotic figures were not observed to account for this proliferation. Moreover, if one cell could form compact cytoplasm, other cells could also. A second view is more in line with microscopic studies. The plastin cells may be derived from mesenchyme cells of the oncosphere, whose granular cytoplasm becomes compact”. He says later: „The real difference, therefore, between somatic and germinative cells breaks down”. In his later papers (1957a, 1957b, 1958, 1959a, 1959b), O g r e n did not distinguish these two types of cells in the embryos studied, which is probably the result of his above conclusion that there is no important difference between them. It should, however, be pointed out that these two types of cells are always visible in the drawings given by him.

The results of O g r e n ’ s 1956a observations concerning the cytological changes in the final phase of *Mesocestoides corti* development are so much in accordance with the results obtained in my studies on *D. ransomi*, that an analysis of his interpretation seems justified. My own observations of *D. ransomi* showed that the plastin cells are formed — in accordance with O g r e n ’ s first, „logical” as he himself says, interpretation — from similar large cells („germinative” after O g r e n). At the final phase of development in both species discussed the same considerable cytoplasmic changes in these cells were observed. The previously granular cytoplasm became a compact one. The increase in the number of plastin cells in *D. ransomi* can be explained by the mitotic figures observed up to the final preoncosphere stage. On the other hand, the decrease in the number of small (mesenchymatic) cells also observed by O g r e n can be satisfactorily explained by their degeneration observed in *D. ransomi* which led to a reduction in the total number of embryonic cells (from 80 to 26). It can be assumed that an analogous process took place in *M. corti*. O g r e n 1956a did not give exact data as to the number of cells in the final stage of embryonic development, only making a general reference to the increase or reduction of the various types of cells.

The cytological changes in the embryo during the preoncosphere phase of *D. ransomi* are connected with cytochemical processes and first of all, the ribonucleic acid distribution. During the degeneration of the small cells this acid disappears in their cytoplasm. At the same time, a large quantity of RNA accumulates in the cytoplasm of the plastin cells. Recent cytochemical research (Brachet 1955) has shown that the amount of ribonucleic acid present in the cell is connected with protein synthesis. There is always a large

accumulation of RNA in the cells where intensive protein synthesis is taking place. Studies on the distribution of this acid during the viruses multiplication, as well as the embryonic development of various vertebrates have shown that it plays an important part in the multiplication of cells and in the morphogenesis of the embryo (Brachet 1955). What is known so far of the role of RNA in the cell makes it possible to understand better the changes in the distribution of this acid observed in the embryonic development of cestodes. My own observations showed that in *D. ransomi* RNA accumulates precisely in those cells which multiply intensively. In the early embryos intensive micromere divisions were observed and at the same time their cytoplasm was rich in RNA. In the preoncosphere phase, the micromeres stop dividing, most of them degenerate and this is accompanied by the disappearance of RNA in their cytoplasm. On the other hand, at this phase the previously syncytial cytoplasm of plastin cells begins to concentrate around the nuclei. Large amounts of RNA are accumulated in the cytoplasm. The same structure of plastin cells is retained in the oncosphere. The large accumulation of RNA deposit in the plastin cells of the oncosphere show that these cells have great development potencies.

Only a few researchers have studied RNA distribution in the embryonic cells of other cestode species (including Ögren 1952, 1959a). However, in all descriptions of the oncosphere so far, attention is attracted by the characteristic strong basophilia of the cytoplasm of the plastin (or germinative) cells. This was also very clearly shown in large pseudophyllidean coracidia by Vogel 1929, 1930 and Michajłow 1932, 1933. Assuming that the basophilia is an approximate index of the RNA (protein ratio in the cell — Brachet 1955) it would seem that in other species too, the large oncosphere plastin cells with basophile cytoplasm are the main element of the further development of the metacestode.

Another question for discussion is the development of the oncosphere envelopes. The first delicate membrane appearing around the fertilized egg after its penetration to the uterus was observed already in the earliest studies on the development of cestodes (van Beneden 1881, Saint Remy 1900, Janicki 1906, 1907). Spätlich 1925 emphasized that it was very delicate and not always clearly visible. Silverman 1954 and Douglas 1956 called it a chorion. Bona 1957 called this membrane „membrana vitellina” owing to its vitelline origin. According to him, in *Paricterotaenia porosa* (Rud.) a granular substance, being a secretion of the uterus, also takes part in the formation of this membrane, apart from the oocyte yolk and the vitelline cells.

The observations made did not enable me to identify exactly the chemical composition and the source of the vitelline membrane. The changes observed in the oocyte and vitelline cell after they had entered the uterus and the disappearance of the granular substance found there previously, show that all the three elements mentioned by Bona could go to form this membrane in *D. ransomi*.

According to Bona 1957, this membrane is homologous for the shell of *Trematoda* and *Pseudophyllidea* eggs. According to his observations of *P. porosa*, the vitelline membrane exists all through the development of the embryo and adheres closely to the outer membrane of the oncosphere which later

surrounds the embryo. Bona recognized it in further development stages by the PAS-positive reaction. I have not observed any PAS-positive reaction in membrana vitellina of *D. ransomi*. In the older embryos it became impossible to distinguish this delicate membrane.

Beneath the membrana vitellina, the outer membrane of the embryo appeared. It was formed by the detachment of three macromeres with a thick layer of their syncytial cytoplasm from the mass of embryonic cells. The formation of this membrane is similar, in principle, in all the species examined so far, though some differences in various species were observed. According to Spätlich 1925, the outer membrane in *Diorchis inflata* is formed by only two cells; according to Janicki 1906, it is formed in *Taenia* from three macromeres. Van Beneden 1881 wrote about four cells going to form this membrane in *Taenia*. According to Bona 1957 it is formed by two cells in *P. porosa*. In the opinion of the authors mentioned, the macromeres forming the membrane contain the entire yolk of the embryo. In the later condition the macromeres disintegrate and the products of their disintegration together with the remains of the vitelline cell form the outer membrane. In *D. ransomi* the nuclei of the macromeres remain in the fully formed oncosphere. The share of the vitelline cell in the formation of this membrane could only be recognized by the presence of glycogen in it. The entire amount of glycogen passed from the vitelline cell to the outer membrane.

In *D. ransomi*, the outer membrane is preserved during the whole life of the oncosphere in the uterus, in water and in the intestine of the intermediate host. Similar resistance of this membrane was also observed in *D. inflata* (Spätlich 1925), in *Choanotaenia marchali* (Bona 1956) and in *Paricterotaenia porosa* (Bona 1957). On the other hand, in cestodes of the genera: *Taenia* (Janicki 1906, 1907), *Multiceps* (Johri 1957), *Mesocoeloides* (Ogren 1956a), the membrane, which appears in the early stages of development, decomposes relatively quickly. Bona 1957 puts forward the hypothesis that the longevity of the membrane in various cestodes of water fowl is an example of convergence resulting from adaptation to environment.

The second embryonic envelope is the inner membrane, or embryophore. Owing to its great rigidity, the embryophore is often referred to as a shell. Smyth et Clegg 1959 point out that the use of this term can cause confusion in comparing the eggs of *Cyclophyllidea* and those of *Trematoda* and *Pseudophyllidea*, in which this term means the outer shell with operculum, which, according to Bona 1957, is a homologue of the „membrana vitellina” in *Cyclophyllidea*.

In investigations made so far, the greatest attention has been paid to the embryophore. In the majority of species it is described (van Beneden 1881, Janicki 1907, Spätlich 1925, Silverman et Manely 1955, Ogren 1956a, Bona 1957) as cellular in structure. Bona 1957 gives a very detailed description of the formation of this membrane in *P. porosa*. In that species, this membrane is a homogenous one which later differentiates on the outer layer of the granular cytoplasm („involucro”) and the compact cytoplasmic layer surrounding the embryo in which the disintegrating nuclei of the mesomeres remain. This inner layer thickens later forming the embryophore. The differentiation of the inner membrane on the aforementioned two layers often leads, in Bona's opinion, to it being erroneously treated as two separate

membranes, owing to which authors write that three membranes appear in the embryo (outer, inner and embryophore). The proportions of the two parts of the inner membrane (the embryophore and the granular layer) are different in different species. In some the entire membrane becomes the embryophore, while in others (*Paricterctaenia porosa*, *Choanetaenia marchali*, some *Anomotaenia* species of water fowls — B o n a 1956, 1957) the granular layer is preserved in the mature oncosphere as an intermediate membrane between the embryophore and the outer membrane. In this group, B o n a 1957 includes also *Diorchis inflata*. According to data given by Spätlich 1925 and Rybicka 1957a there is no such layer on the outside of the embryophore of *D. inflata* and *D. ransomi*, yet there is a delicate oncosphere membrane formed between the embryophore and the oncosphere, closely adhering to it.

O g r e n 1957a distinguished two types of embryophore in *Cyclophyllidea*. The first of them, observed in the above mentioned species, is a cellular one formed from the epidermal cells of the embryo — he calls this type the embryophore. The second type is the homogenous colloid secretion of the epidermal glands which makes a rigid capsule called by O g r e n the pseudoembryophore. The pseudoembryophore was observed in *Oochoristica*, *Hymenolepis* and *Choanotaenia* (O g r e n 1957a, 1955a, 1955b).

In connection with the differences in the origin of the embryophore, O g r e n 1957a distinguished two groups among *Cyclophyllidea*:

(1) tapeworms forming a pseudoembryophore. In this group the epidermis of the embryo is retained as the cortex of the oncosphere;

(2) tapeworms forming an embryophore (or ciliaphore). Here, the epidermis separates from the inner cell mass of the oncosphere and becomes the embryophore.

According to my own observations the embryophore of *D. ransomi* is not of cellular structure, which agrees with O g r e n 1957a according to which *Hymenolepididae* belong to the group forming a pseudoembryophore. On the other hand, this observation does not agree with the data given by Spätlich 1925, who is of the opinion that the embryophore of *D. inflata* is a cellular one.

Little is known so far about the chemical composition of the embryophore. O g r e n 1957a describes the pseudoembryophore in *Oochoristica* as a colloid protein secretion of the epidermal glands, which, as a protein gel, forms a rigid capsule. However B o n a 1957 maintains that in *P. porosa*, where the epidermal glands were thoroughly investigated during embryonic development, they do not play any part in the formation of the embryophore. Their role, after Silverman et Manely 1955, is to facilitate the penetration of the oncosphere through the intestine wall of the intermediate host. The function of the epidermal glands as penetration glands is also confirmed by the studies of Enigk et Sticinsky 1957 on *Davainea proglottina*.

The histochemical studies made by Johri 1957 suggest that the embryophore in *Multiceps Smythi* Johri is a keratin type of protein. The author points out that the term „keratin” is only loosely used to include the content of certain types of proteins. Johri also studied the formation of the embryophore. According to his histochemical analyses, the vitelline cells and the ova contain large bodies believed to be acidophile proteins and it is possible that these bodies play some part in embryophore formation.

Beneath the embryophore was the oncosphere membrane also distinguished by Spätlich 1925 in *D. inflata*. In his studies on *P. porosa*, Bona 1957 observed beneath the embryophore the epithelium of the oncosphere, which later changed into the cuticula. At the same time, the author makes the assumption that the third membrane (inner membrane) in *Diorchis* described by Spätlich 1925 is also an epithelium. This is a very difficult problem to solve as the aforementioned membrane is barely distinguishable and adheres closely to the oncosphere, which would point to its epithelial character.

Observations concerning glycogen distribution during the development of the embryo of *D. ransomi* were previously discussed in detail by me (Rybicka 1960). A comparison of investigations of glycogen distribution in cestodes enabled me to distinguish the following groups:

1. Cestodes containing glycogen in the vitellarium. The vitelline cell containing glycogen enters the uterus together with the egg and participates in the formation of the embryo. *D. ransomi* belongs to this group.

2. Cestodes without glycogen in the vitellarium and in the early embryonic stages.

Among the tapeworms of group 1, changes in the glycogen distribution in the final phase of development were examined thoroughly in *Hymenolepis diminuta* and *Raillietina cesticillus* (Hedrick et Daugherty 1957). It is interesting that the changes in glycogen distribution observed in *D. ransomi* are identical to the changes observed in *R. cesticillus* and different from those in *H. diminuta*, in spite of the close relationship between *Hymenolepis* and *Diorchis* genera.

LARVAL DEVELOPMENT

Results

The primary aim of the present studies was to observe the cytological changes in the first phase of larval development.

The larvae of experimentally infected *Ostracoda* were pressed out at various intervals of time following infection. Oncospheres pressed out 1/2 hour after feeding the host with tapeworm eggs were still in its intestines in the egg envelopes, but they differed definitely from the oncospheres observed before entering the intermediate host. In the uterus of the tapeworm or in water after having been pressed out from the uterus, the oncospheres were always immobile, of unchanging spindle-like shape with immobile hooks in their permanent position. In the intestine of the ostracode, the oncosphere began to make violent movements flexing and unflexing and moving round inside the embryophore, at the same time making intensive movements with the hooks. The shape of the oncosphere changed from spindle-like to spherical or slightly oval (Fig. 6).

The movements of the oncosphere showed that its hatching was not the result of the digestion by the host of its envelopes, but of its active movements to emerge.

Fig. 6 shows three oncospheres pressed out from the intestine of the host 1/2 hour after its infection and fixed in various stages of its emergence from the envelopes. The cytological structure of these oncospheres did not differ

from the structure of the oncospheres in the uterus of the tapeworm. In the hook region micromere nuclei were visible, the whole body was filled with clearly differentiated plastin cells.

After entering the hemocoel of the host, the larva began to grow very quickly. At this stage its plastin cells grew and multiplied intensively. It was very difficult to establish the exact number of cells in the successive stages of further development because only total larval preparations were studied and this is why only approximate cell numbers are given in descriptions of further development.

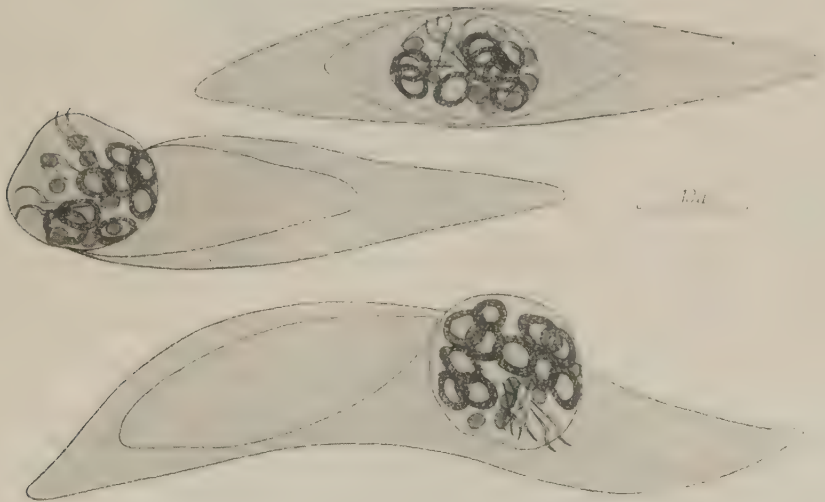


Fig. 6. Three oncospheres during hatching (Methyl green/pyronin)

A spherical larva, 50 μ in diameter (Fig. 7), had over 30 cells about 10–12 μ in diameter. These cells had large (about 6 μ), slightly basophile spherical nuclei. The nuclei were surrounded with a thick layer of strongly basophile vacuolated cytoplasm. The structure of the cells was very similar to that of the plastin cells of the oncosphere, differing from them by their larger dimensions. There is no doubt that they were derived from the plastin cells. At this phase, the embryonic hooks were still situated relatively near each other. Near the hooks, small (1.7 μ) nuclei could be seen, strongly stained with methyl green. The cytoplasm of these small cells was colourless and the cell boundaries were not seen. The number of small nuclei observed in the larva at this stage was about 11. Nine of them were near the hooks and one at each side of the body. Both the appearance of these nuclei and their location showed that they were the unchanged nuclei of the oncosphere small cells.

In a larva 65 μ in diameter (Fig. 8) the number of previously observed large cells was as many as 100. Their structure did not differ from that of the cells described above. On the other hand, at that stage no micromeres situated near the hooks in the body of the larva could be found. The hooks were loosely scattered over the surface of the larva.

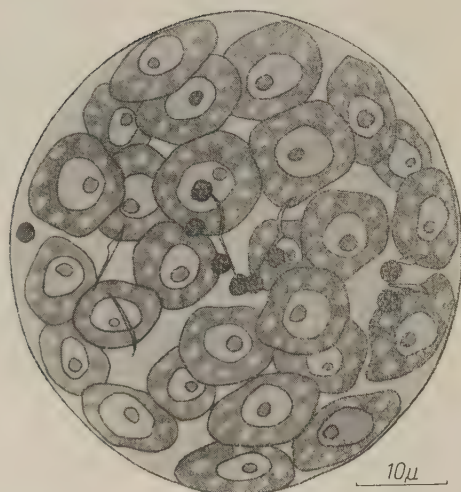


Fig. 7. Larva of over 30 cells (Methyl green/pyronin)

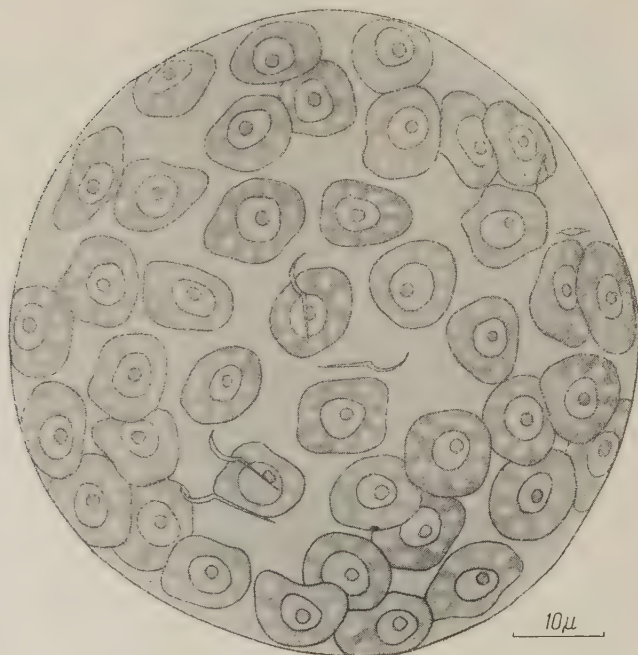


Fig. 8. Larva of over 100 cells (Methyl green/pyronin)

The disappearance of the micromeres showed that the further development of the larva took place by multiplication of the large cells — derived from the plastin cells of the oncosphere.

The growing larva kept its spherical shape for a certain time. It should be pointed out that the living larva changed its shape slightly at this stage, becoming more elongated and narrower (as in Fig. 3b, Rybicka 1957), but after fixation it always assumed a spherical shape.

When the larva was about 125 μ in diameter, the number of cells was over 300. In that phase differentiation of cells was observed. Some of them were similar to those observed previously, but they were slightly smaller in diameter (8—9 μ). The others were smaller ones of varying diameters (3.3 to 5.5 μ). The spherical nuclei of these cells were surrounded by a thin layer of basophile cytoplasm. These cells looked like the germinative-somatic cells described by Wiśniewski 1930 and Michajłow 1932.

In this stage as in the previous one, the small micromere nuclei of syncytial cytoplasm were not observed in the larva.

In its further development, the larva, so far spherical, grew longer. At this stage, when it measured 315 $\times$ 160 μ , definite cytological changes were observed. The cells were concentrated at the polar end where the cercomers formed were rather large (8.5 μ), and a few slightly smaller cells were visible between them. The tissue of that end of the larva was clearly differentiated from the rest of its body. All the cells were loosely distributed, there being a lot of granular material in the intracellular space. Both the cytoplasm of the cells and the intra-cellular material were slightly basophile.

The body of the larva was composed of cells with strongly basophile cytoplasm. Two types of cells described in the previous, spherical phase could be differentiated. The dimensions of the large cells underwent changes. These cells got smaller at each successive dividing and the layer of compact cytoplasm surrounding them got thinner. This cytoplasm was very compact and not vacuolated. The large cells were mostly located on the surface of the larva while the previously described cells with the thin layer of cytoplasm filled its inside. It should, however, be pointed out that this description of the location of the cells is very rough as it was based on investigations of total preparations. Near the front part of the body of the larva, at the base of the future rostellar bulb, a large agglomeration of small cells about 2.5 μ in diameter was observed. They had a very thin layer of cytoplasm and their nuclei were relatively strongly stained with methyl green.

In the next stage of development (Fig. 6b, Rybicka 1957b) the larva underwent morphological differentiation. The scolex was formed, and on it the anlagen of the suckers and the rostellum. Behind the scolex the body got narrower — the future neck of the cysicercoïd, and behind this, the anlagen of the cyst and the cercomer. The cytological structure of the larva observed in total preparations at this phase gave the impression of being more homogenous. However the larva composed of many layers of cells one upon another, made it impossible to state with certainty whether there was greater cytological differentiation than that which could be observed. The diameter of the cells at this stage was about 3.5 — 4 μ . The cytoplasm of these cells was very thin and the nuclei were intensively stained with methyl green.

The cells of the cercomer were large, slightly stained with pyronine, and their boundaries were not clearly visible. Between the cells there was a lot of the previously observed granular material.

The cytological structure of the already formed cysticercoïd before its incystation underwent further changes. The boundaries of the cells became less clear and the body of the larva seemed to be a syncytial mass. The cytoplasm was stained with pyronin. At this phase, the nuclei were small (about $2\ \mu$), compact and intensively stained with methyl green. The dimensions of these cells and the fact that their nuclei were colourless reminds one rather of the micromeres observed in the oncosphere, which could lead one to assume that the cells of the fully formed larva were derived from the oncosphere micromeres. This assumption was however contradicted by the degeneration of the micromeres observed previously and their complete disappearance in the early stages of larval development. The cells of the cysts showed clearly differentiated boundaries about $3.5-5\ \mu$ in diameter. The nuclei of those cells were slightly basophile.

The structure of the cercomer was syncytial. Both the cercomer cytoplasm and its loosely distributed spherical nuclei $3-5\ \mu$ in diameter were slightly basophile.

Discussion

Cytological studies on the early larval development of *Diorchis ransomi* showed that after the oncosphere had entered the hemocoel of the intermediate host it grew very quickly owing to the growth and the multiplication of the platin cells. The nuclei of the small cells in the hooks region disappeared in the early stage of larval development. The hooks helped the oncosphere in boring through the intestinal wall of the host and passing to its hemocoel. They did not play any part in the further development of the larva and later moved on to the cercomer. The location of the small cells in the region of the hooks and their disappearance at the time the function of the hooks ended, leads one to assume that the role of these cells in the oncosphere is only connected with the function of the embryonal hooks.

The observations of the fate of both types of cells during larval development supported the conclusions drawn on the basis of the studies on embryonic development. The accumulation of large reserves of RNA in the cytoplasm of the oncosphere platin cells showed their considerable developmental potencies, and this was reaffirmed by observations of larval development. The disappearance of RNA in the cytoplasm of the small cells of the embryo was connected with their degeneration, begun in the preoncosphere phase, which led to their final disappearance in the larva.

The fate of both types of oncosphere cells in the larval development of the tapeworm has been studied so far in only few species. The data given by various authors are not always in agreement with each other. Wiśniewski 1930 distinguished small „somatic cells” and large „germinative cells” in the oncosphere of *Archigetes*. The parenchyma, nervous system, excretory system and genital ducts of these cestodes were formed in his opinion from the „somatic cells”. The „germinative cells” were differentiated into two types, the

so-called „germinative-somatic cells”, which also play a part in the parenchyma formation, and the true „germinative cells” which are the anlagen of the reproductive glands. Michajłow 1932, 1933 observed both of the aforementioned types of cells in the coracidium of *Triaenophorus*. According to Michajłow, in the development of the proceroid, the „somatic cells” continued to divide, forming the parenchyme of the proceroid while the „germinative cells” differentiated giving several new types of cells, among others „the germinative-somatic cells”. Vogel 1929, 1930 found the same types of cells in the coracidium of *Diphyllbothrium latum*. He called the small cells „somatic” or „Körperzellen” and the large ones „plastin cells”. In his opinion, the „somatic cells” did not participate in the further development of the proceroid and were only connected with the function of the embryonal hooks. The larva was formed from the „plastin cells” which, as it developed, underwent further changes. Cytological studies on larval development were also carried out by Ogren 1957b on *Oochoristica symmetrica* Baylis. According to his observations, in the early development of the larva „... the major activity consisted in the transformation of granular oncosphere cells into compact plastin or germinative cells” (p. 152). As Ogren used the name „granular cells” to define both small and large embryonal cells, it is difficult to tell which type he was referring to.

This comparison shows how different authors gave different interpretations of the two types of cells in the oncosphere, and their further fate during larval development.

My observations show that the larva of *D. ransomi* as well as the adult cestode develop from the plastin cells of the oncosphere. A strong basophilia of plastin cells and its disappearance in somatic cells, observed in oncospheres of all species studied so far, seem to show that the plastin cells are being the anlagen of the adult cestode.

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STRESZCZENIE

Celem pracy było zbadanie rozwoju tasiemca *Diorchis ransomi* Schultz 1940, w oparciu o metody cytologiczne i cytochemiczne.

Badanie gametogenezy wykazało, że cały plemnik (główka i wić) powstaje z jądra spermatydy.

W badaniu rozwoju embrionalnego prześledzono: a) wczesne fazy rozwoju zarodka, b) fazę preonkosfery wyróżnioną przez autorkę, c) fazę onkosfery. We wczesnych fazach rozwoju wyróżniono trzy typy blastomerów tworzące początkowo zespół syncytialny. Cytoplazma młodego zarodka zawiera RNA. Trzy makromery stosunkowo wcześniej oddzielają się od reszty komórek wraz z grubą warstwą cytoplazmy, która tworzy osłonkę zewnętrzną zarodka. We wczesnych fazach rozwoju najintensywniej mnożą się mikromery. Maksymalna liczba komórek zarodka wynosi ok. 70.

Kończącą fazą rozwoju — preonkosferę charakteryzują następujące procesy:

1) Zmiana postaci zarodka i jego osłonki zewnętrznej, dająca typową dla gatunku postać onkosfery i „jajeczka”.

2) Wytworzenie embrioforu między osłonką zewnętrzną i zarodkiem.

3) Degeneracja części mikromerów zarodka, dająca znaczne zmniejszenie liczby jego komórek.

4) Pojawienie się wyraźnie zróżnicowanej warstwy cytoplazmatycznej dookoła jąder makromerów, które przybierają postać komórek plastinowych.

5) Zmiany rozmieszczania RNA w komórkach zarodka (zanik RNA w cytoplazmie mikromerów i nagromadzenie się tego kwasu w cytoplazmie mezomarów).

6) Pojawienie się w zarodku nieglikogenowej substancji PAS pozytywnej.

Onkosfera stanowi postać utrwaloną morfologicznie do czasu przejścia do żywiciela pośredniego. Otoczona jest osłonką onkosfery, embrioforem i osłonką zewnętrzną. Liczba komórek onkosfery wynosi 26. Wśród nich można wyróżnić położony w rejonie haków zespół komórek somatycznych, zawierający 9—13 małych, silnie bazofilnych jąder. Cytoplazma komórek somatycznych nie wykazuje obecności RNA. Poza tym widoczne jest 12—16 dużych, wyraźnie zróżnicowanych komórek plastinowych. Silnie bazofilna cytoplazma tych komórek zawiera duże zapasy RNA.

Analiza procesów zaobserwowanych w czasie rozwoju zarodka wykazała, że intensywny rozwój mikromerów we wczesnych fazach rozwoju związany jest z dużym nagromadzeniem RNA w ich cytoplazmie. Degeneracja tych komórek w fazie preonkosfery wiąże się z zanikiem u nich RNA. W fazie preonkosfery duże zapasy RNA gromadzą się w jej komórkach plastinowych, co wskazuje na ich duży potencjał rozwojowy.

W początkowym okresie rozwoju larwalnego zanikają komórki somatyczne onkosfery. Komórki plastinowe rosną bardzo szybko i ulegają intensywnym podziałom, tworząc ciało larwy. W dalszym rozwoju komórki te ulegają zróżnicowaniom morfologicznym.

Los obu typów komórek w czasie rozwoju larwalnego potwierdza wnioski wyprowadzone na podstawie badania rozwoju embrionalnego. Intensywny rozwój komórek plastinowych u larwy związany jest z nagromadzeniem dużych zapasów RNA w tych komórkach już u onkosfery. Zanik RNA w komórkach somatycznych wiąże się z ich degeneracją rozpoczętą w fazie preonkosfery i zakończoną w początku rozwoju larwalnego.

Obserwowana u wszystkich poznanych dotychczas onkosfer tasiemców silna bazofilia komórek plastinowych i zupełny jej brak w komórkach somatycznych pozwala przypuszczać, że u wszystkich gatunków tylko komórki plastinowe stanowią zawiązek ciała larwy a tym samym i tasiemca dorosłego.